Introduction

Purpose

This document describes the guidance and procedures for the national surveillance program for bovine spongiform encephalopathy (BSE) beginning in June 2004. The goal of the program is to test as many cattle as possible in the described targeted high-risk population over a 12-18 month period along with a sample of normal aged animals presented at slaughter. At the end of this period, APHIS and FSIS will evaluate the results and adjust future policies based on the evaluation.

The purpose of this document is to clarify:

- The objective of the overall survey and the objective for each of the three categories of animals to be sampled:
  - Cattle highly suspicious for BSE
  - Targeted high risk cattle
  - Normal adult cattle
- When to refer a highly suspicious for BSE animal to the Area Veterinarian-In-Charge (AVIC) for a possible Foreign Animal Disease (FAD) investigation
- Personal safety guidelines
- When and how to sample targeted high risk cattle
- When and how to sample apparently normal adult cattle
- What information to record about the sample
- How to ship the sample
- Where to ship the sample
- Communication protocols
- Disposal of the carcass and offal

In addition, a number of appendices give more detailed information:

If you need:	**Then see appendix**
VS Memo 580.16 on BSE surveillance procedures	A
The entire national BSE Surveillance Plan	B
Example of a state-specific plan	C
One page summary of the sampling procedures	D
Guidance on what a quality sample looks like	E
Contact information for APHIS, VS Area-Veterinarians-In-Charge	F
Instructions on filling out paper BSE submission forms and the Web versions of those forms	G
Information about the use of barcodes in shipping your sample	H

If you need:	**Then see appendix**
Information on determining the age of cattle	I
General background information about BSE	J
FSIS documents	K
Information USDA has sent to the media and made available to the public	L
Information about how FAD investigations are conducted	M

Surveillance Plan Overview

The Animal and Plant Health Inspection Service (APHIS), in cooperation with the Food Safety and Inspection Service (FSIS), and the Food and Drug Administration (FDA), has begun an intensive national BSE surveillance plan to determine whether BSE is actually present in the cattle population and if so, at what level. The goal is to test as many targeted high risk cattle as possible in a 12-18 month period. The three agencies will analyze the results and consider future actions. This plan also incorporates random sampling of clinically normal aged cattle at slaughter.

There are 3 categories of cattle to consider: 1) cattle highly suspicious for BSE, 2) cattle in the targeted high risk population, and 3) normal adult cattle presented for slaughter. Here are the surveillance objectives for those three categories:

1) Cattle Highly Suspicious for BSE

Cattle fit this category if the clinical signs of BSE are observed as described in Veterinary Services (VS) Memorandum 580.16 (Appendix A). These observations are usually made on the farm or at other locations where animals can be observed over a period if time. According to Office International des Epizooties (OIE) guidelines, with an estimated adult cattle population of 45,000,000 in the U.S., the minimum number of cattle in this category in 12-18 months is approximately 450.

All cattle that fit this category should be referred to the AVIC in your state (see Appendix F for a list of AVIC offices and contact information, or go to http://www.aphis.usda.gov/vs/ncie/pdf/vsavic.pdf). The AVIC or their designee will determine if a full FAD investigation is warranted and assign a Foreign Animal Disease Diagnostician (FADD) to collect a BSE sample. Note that the procedures for collecting a sample as part of an FAD investigation are different from the regular surveillance sampling procedures.

See Appendix M for a copy of VS Memorandum 580.4 that provides the guidance for conducting FAD investigations. The VS Memorandum 580.16 contains the details of the procedures for taking a sample as part of an FAD investigation.

2) Cattle in the Targeted High-Risk Population

The objective for this portion of the plan is to collect samples from as many adult cattle from the targeted high-risk population as possible in 12-18 months with an appropriate geographical representation. See pages 8 to 23 for instructions on collecting a sample.

3) Apparently normal adult cattle presented for slaughter

Plans are being developed for sampling several thousand adult, clinically normal slaughter cattle. The samples will be obtained from cattle going to the largest adult cattle slaughter plants, which handle approximately 86 percent of the adult cattle slaughtered annually in the United States. The largest volume plants are located in AZ, CA, GA, ID, MI, MN, MO, NC, NE, OH, PA, SC, SD, UT, TX, WA, and WI.

Sampling the Targeted High-Risk Cattle for BSE

If cattle are **not** highly suspicious for BSE yet show some of the signs described below, they are part of the targeted population for this survey:

Definition – Targeted Cattle Population

Age – Unless otherwise designated, samples should only be obtained from animals over 30 months as evidenced by the eruption of at least one of the second set of permanent incisors. (See Appendix I for instructions on how to determine the age of cattle.)

Clinical Presentation Criteria

1. Downer / nonambulatory cattle – Cattle that cannot rise from a recumbent position (downer) or that cannot walk including, but not limited to, those with broken appendages, severed tendons or ligaments, nerve paralysis, fractured vertebral columns, or metabolic conditions, as well as cattle that are severely weakened though they may be able to stand and walk for brief periods of time.

2. Central nervous system (CNS) signs and/or rabies negative – sample animals of any age:
   a. Diagnostic laboratories – samples submitted due to evidence of CNS clinical signs.
   b. Public health laboratories – rabies negative cases.
   c. Slaughter facilities – CNS antemortem condemns at slaughter, sampled by FSIS
   d. On-the-farm – CNS cattle that do not meet the criteria for an FAD investigation.

3. Cattle exhibiting other signs that may be associated with BSE – Cattle that were condemned or euthanized or that died as a result of a moribund condition, tetanus, emaciation, injuries, or non-ambulatory conditions.

4. Dead cattle – Any dead cattle where the specimen is of diagnostic quality and the cause of death and/or clinical signs prior to death, if known, do not preclude it from the targeted population.

Collection sites

Samples can be collected most efficiently and cost-effectively at concentration points. These are facilities where multiple animals or carcasses are collected, such as a rendering facility, a deadstock collection point, or a salvage (3D/4D) slaughter facility. While focusing efforts on these facilities will allow the highest number of samples to be collected within a defined time frame, samples may be collected at any site where targeted animals are located. In addition to those previously identified, this may include on the farm, state or federally inspected slaughter facilities, livestock markets, veterinary clinics, diagnostic laboratories, or any other facility as necessary.

There are special instructions for cattle condemned on ante-mortem inspection at slaughter:

Cattle condemned on ante-mortem inspection at slaughter (See Appendix K, page K14):

All cattle, regardless of age, condemned by FSIS upon antemortem inspection for CNS impairment will be sampled.

All cattle, with the exception of veal calves (less than 400 pounds at slaughter,) condemned by FSIS upon antemortem inspection for any reason other than CNS will be sampled.

This sampling will be done on-site at the slaughter establishment unless documented, verifiable alternative arrangements for off-site sampling have been approved in advance. Sampling done on-site at the slaughter establishment will be done by FSIS personnel at federally inspected plants, and either APHIS or state personnel at state inspected plants. Sampling done through approved off-site arrangements will be done by APHIS personnel or their contractors.

Samples will be collected from these animals and submitted to the designated laboratory regardless of sample quality. Results from testing of cattle in this category will be provided; however, unless the samples are from cattle that meet the above definition of targeted samples, these data will not be included in the statistical analysis nor will they count toward our BSE surveillance sampling target.

Sampling Apparently Normal Adult Cattle Presented for Slaughter

Plans for this surveillance are still being finalized. Current draft protocols call for a total of 20,000 samples to be obtained from apparently normal adult (over 30 months old) cattle presented for slaughter at the largest adult cattle slaughter facilities. These facilities slaughter most of the adult cattle in this country.

Procedures for Obtaining and Submitting Samples from Targeted High-Risk and Apparently Normal Cattle

- ☐ **Collect the brain stem, including the obex.** Use a brain tissue spoon or other suitable device. Sampling spoons and tools will be provided by NVSL to sample collectors.

- ☐ **Prepare samples for shipping**. Sample collectors must evaluate the acceptability of the tissue sample. Samples that are taken from the wrong location or that are significantly autolyzed are not testable, and should not be submitted unless specific arrangements are made in advance. The only exception to this is for samples taken from cattle condemned as a result of an antemortem inspection. See Appendix E for guidance on taking and submitting a quality sample.

- ☐ **Complete forms for sample submissions.** Sample submitters must accurately record all relevant information on the USDA BSE Surveillance Submission Form, USDA BSE Surveillance Submission Continuation Form if used, and on each of the USDA BSE Surveillance Data Collection Forms. Enter this information on the electronic version of these forms – either on a tablet PC or via the web-based forms – unless such electronic entry is impossible. Print a copy of the completed USDA BSE Surveillance Submission Form, USDA BSE Surveillance Submission Continuation Form if used, and on each USDA BSE Surveillance Data Collection Form to accompany the samples shipped to the designated laboratory. If the BSE Surveillance Submission Form is submitted electronically, the submitter should keep a hard copy of the form and each BSE Surveillance Data Collection Form and retain those on-site.

- ☐ **Submit samples and corresponding paperwork to the designated laboratory for your location.** Refer to the list of designated laboratories in the attached table (see pages 21-22).

☐ **Collect all animal identification devices, brands (via digital picture or drawing), and tattoos (refrigerate tissue containing tattoo) from each animal sampled.** Bag these identification items, label them with the sample number and bar code sticker, attach a copy of the USDA BSE Surveillance Submission Form, and save until negative results are received. If samples are obtained for other disease programs (i.e., tuberculosis) from the same animal, record the animal identification on the appropriate submission form and indicate on the form where the identification items are stored. When whole carcasses are held, the ID may be stored on the carcass until results are returned. All parts of a stored carcass should be identified with an appropriate device.

☐ **Distribute and file copies of the completed sample forms.** Provide copies to the VS Area Office, the collection site, one set to be maintained with the identification devices, and one set for the sample submitter's file. Samples for which appropriate information is not recorded will not be counted towards the target. Samples from animals which do not meet the listed criteria will not be counted towards the target. These samples will be tested, but they will not contribute to the overall numbers.

☐ **Notify the designated laboratory of the incoming samples.**

☐ **Confirm with the overnight contract delivery service that the samples were delivered to the laboratory.**

☐ **Designated laboratories will report "not detected" or "inconclusive" screening test results back to the sample submitter, the AVIC, and when requested, to plant management of the facility where the sample was collected.** Rapid screening test results will be reported as either "not detected" or "inconclusive," in accordance with VS Memo 580.16 (Appendix A).

Personal Safety

If BSE is transmissible to humans in the occupational setting, the most likely routes would be through contact with infective tissues through wounds or open lesions on the skin, contact with mucous membranes (eyes and mouth), or exceptionally, by swallowing. Transmission by the airborne route (i.e., by the inhalation of infectious airborne particles) is considered to be the least likely route of exposure. In naturally BSE affected cattle, the only tissues that have shown infectivity are the brain, retina, and spinal cord. In experimentally (orally) affected cattle, the distal ileum has also shown infectivity.

Because rabies, listeriosis, and other possible zoonotic diseases must be included in the differential diagnosis, brain and spinal cord collection from cattle with CNS clinical signs should be done carefully. The following precautions are generally applicable:

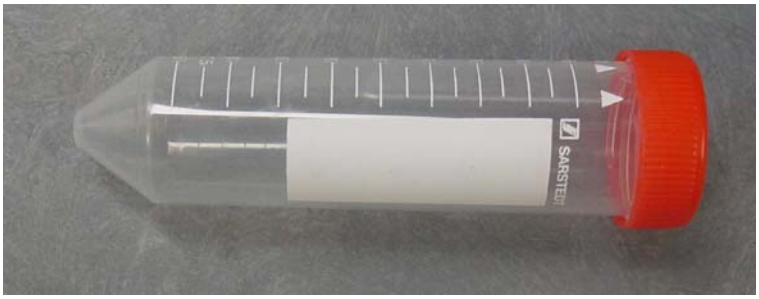
- Adhere to safe working practices and take extra precautions to avoid or minimize the use of tools and equipment likely to cause cuts, abrasions, or puncture wounds.
- Where use of such equipment is unavoidable, wear suitable protective clothing which includes disposable coveralls, aprons, heavy gloves and boots.
- Cover existing cuts, abrasions, and skin lesions on exposed skin with waterproof dressings.
- Use face protection such as a facemask and face shield or goggles to protect the mucous membranes of the eye, nose, and mouth from exposure to infective droplets or tissue fragments.
- Take steps to avoid the creation of aerosols and dusts when engaged in activities such as sawing through the skull bones.
- Wash hands and exposed skin before eating, drinking, smoking, taking medication, using the telephone, or going to the toilet.
- Wash and disinfect protective clothing and instruments thoroughly after use.

Detailed Sampling Procedures

These are the step-by-step procedures for sampling. For a one-page summary, see Appendix D.

Tools needed

- Knife and scissors
- Spoon (or other suitable device)
- Forceps
- Screw top plastic tubes (50ml)
- Fine point permanent marker
- Ball-point pen
- Pan or bucket for disinfecting instruments and rinsing gloved hands
- Bleach (disinfectant)
- Paper towels
- Trash bags
- Supply of BSE mailers (including frozen cold packs)
- May need scabbard, a steel and personal protective equipment



Getting a Sample of Sufficient Quality

Unless the sample is of sufficient quality, it will be unusable and not count towards the survey. Please see Appendix E for guidance on collecting a quality sample. If the sample is not of sufficient quality, STOP: DO NOT TAKE THE SAMPLE. This does NOT apply to samples taken from:

- animals that are highly suspicious for BSE or that involve an FAD investigation
- animals that were condemned in an antemortem inspection

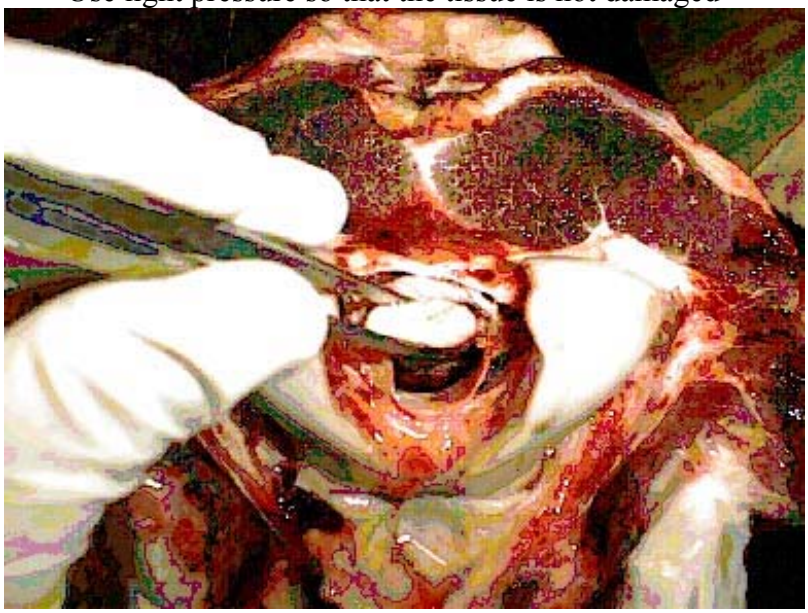
BSE sampling using a spoon

Step 1

- Place head upright
  - On head rack or barrel
  - On table edge
  - On the ground facing down if no other option

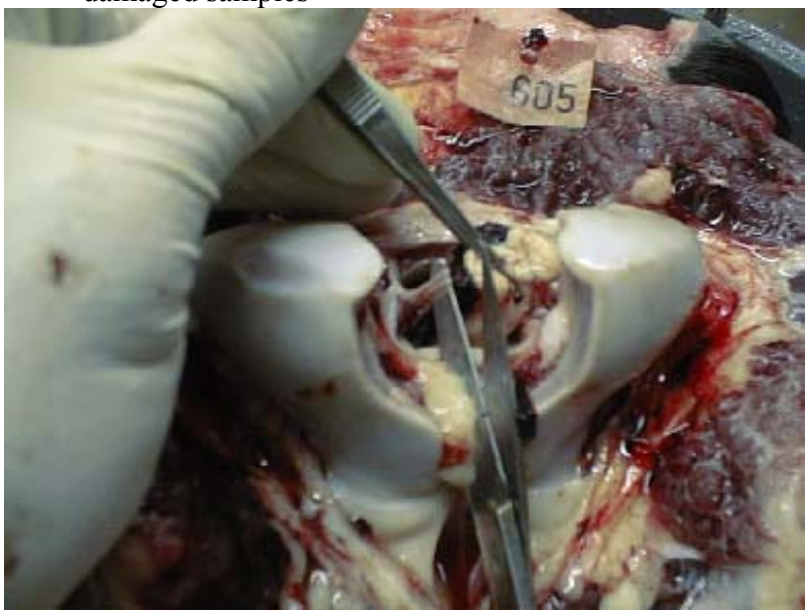
Step 2

- Grasp the spinal cord with forceps
- Use light pressure so that the tissue is not damaged



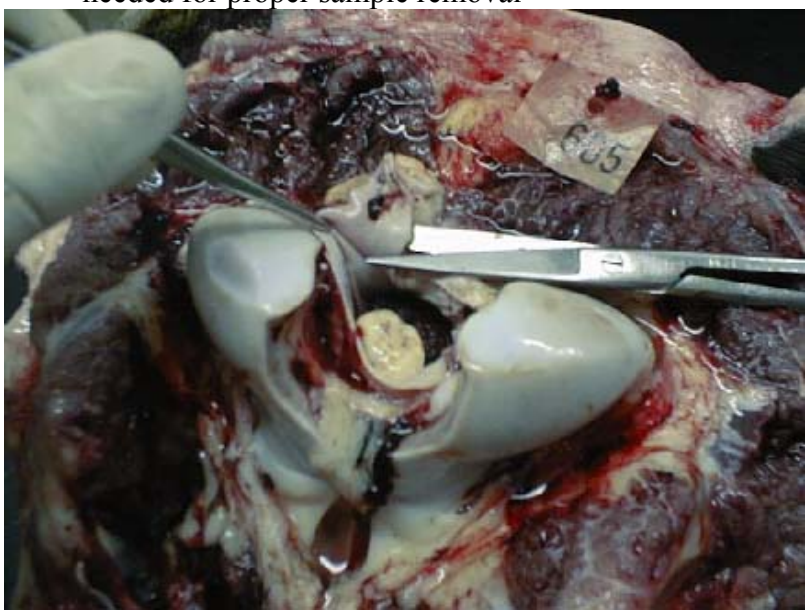
Step 3

- Cut the dura mater & cranial nerves
- Cut down each side of the spinal cord about ½" or more
- Cut on the sides – do not cut into the spinal cord
- Failure to sever cranial nerves is a common cause of damaged samples



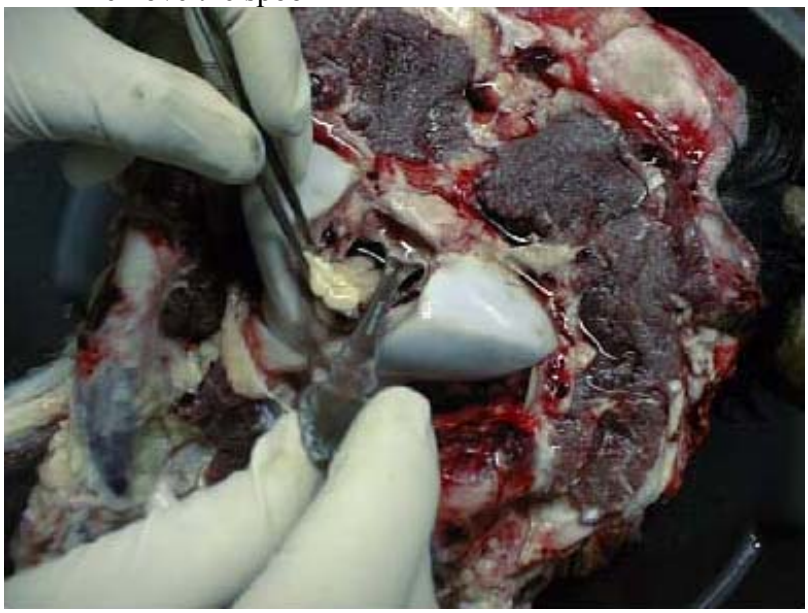
Step 4

- With forceps and scissors remove as much dura mater as possible
- Dura mater removal allows better visualization and is needed for proper sample removal



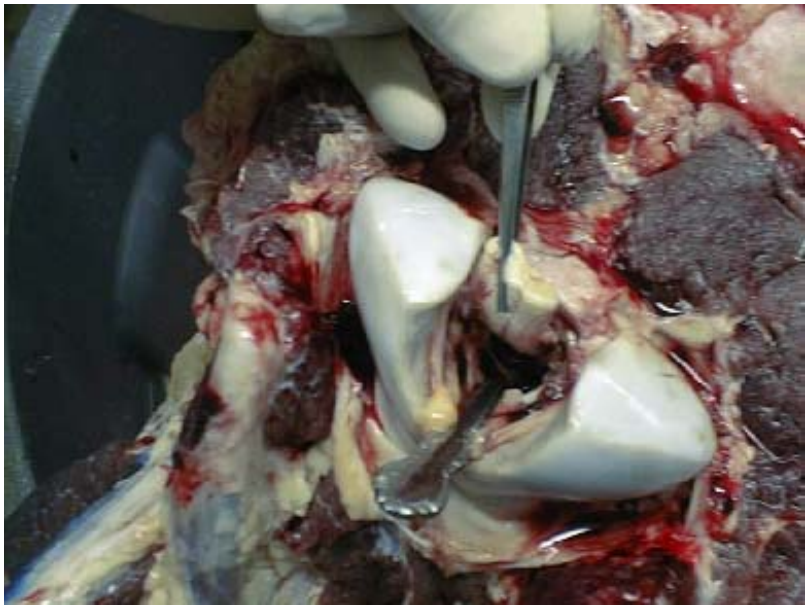
Step 5

- With light pressure use forceps to hold the spinal cord to the ventral part of the foramen
- Insert the spoon (inverted) on the dorsal part of the spinal cord to sever the cerebellum
- Remove the spoon



Step 6

- With forceps lift the spinal cord dorsally and re-insert along the ventral surface of the spinal cord
- Lower the handle of the spoon to sever the cord/brain stem
- With constant upward pressure/dorsal movement of the front edge of the spoon, gently work the severed sample from the foramen

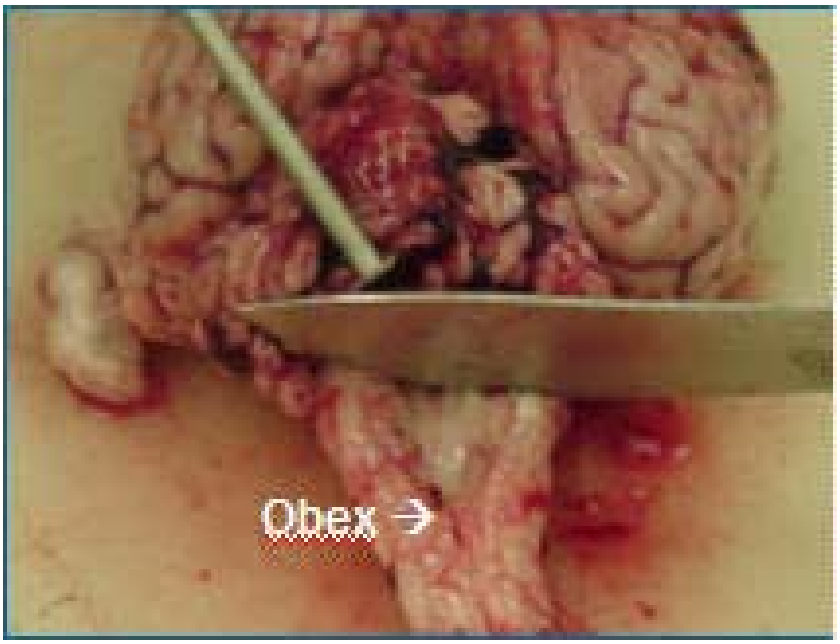


Step 7

- Complete removal of the sample from the foramen
- Clean off excess blood

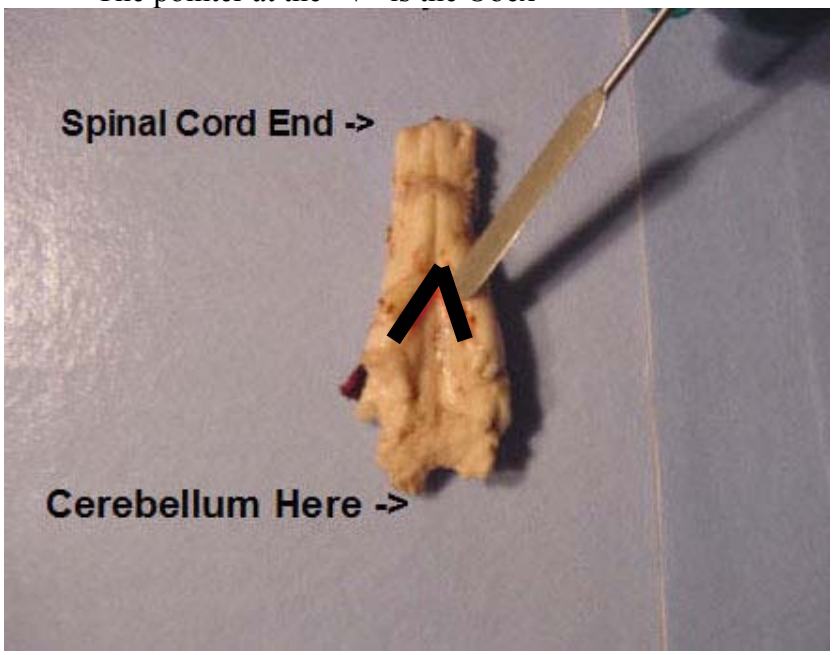
Step 8

- You should be able to identify the Obex area of the brain
- Make sure your samples contain the Obex
- The Obex **MUST** be collected for the sample to be used in BSE the surveillance data



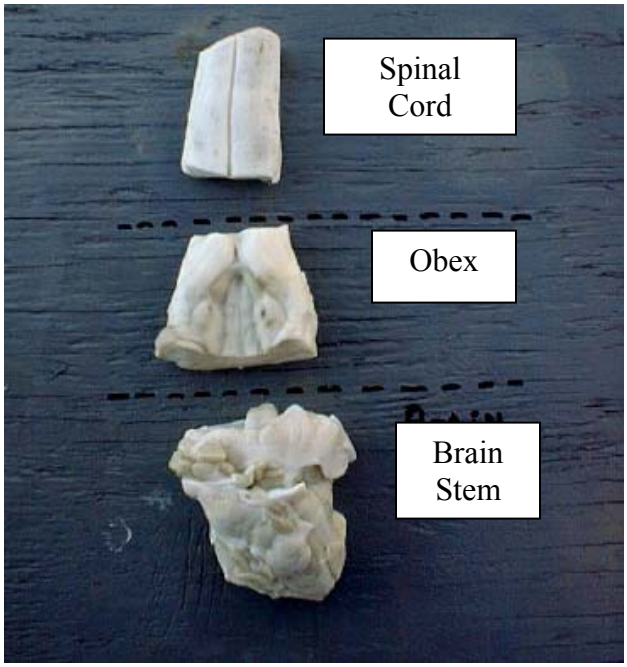
Note:

- The area marked in **black** is the location of the Motor Nucleus of the Vagus nerve
- The nucleus appears as "pink fleshy" areas
- This nucleus is the area we examine in the lab
- The pointer at the "V" is the Obex



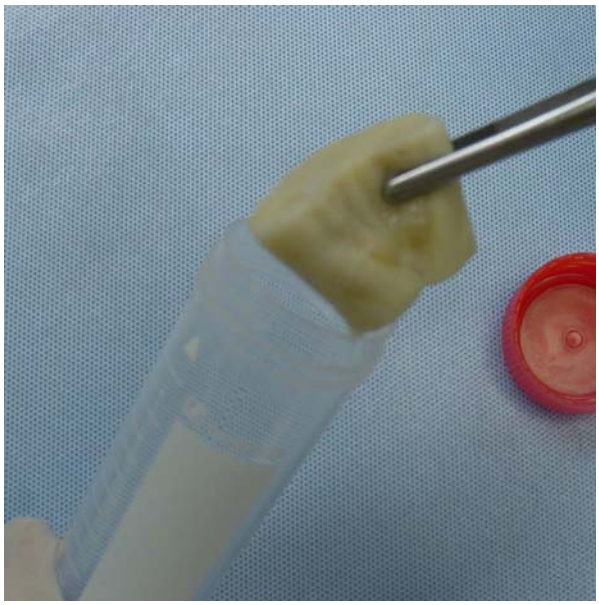
Step 9

- Cut the samples as pictured
- The middle piece of tissue contains the Obex and the Motor Nucleus of the Vagus
- The Obex is the **key area**



Step 10

- Remember the sample will be ***FRESH*** tissue
- **NO** formalin
- Place the Obex in the supplied screw top tube
- Label the sample tube with:
    - Sample number (ex: 1,2,3,4)
    - Barcode ID label
- Dispose of non-submitted tissue with carcass



Shipping the Sample

Packaging Materials (Supplied from NVSL as BSE Kits)

- Approved shipping box.
- Plastic bag or Zip-loc bag to place sample tubes in.
- USDA BSE Surveillance Submission Form.
- USDA BSE Surveillance Data Collection Form.
- Absorbent material.
- Ice packs.
- (2) bio-hazard bags (to comply with the International Air Transport Association (IATA) shipping regulations)
- Labels for shipping regulations compliance (air eligible, IATA statement, UN 3373, Keep from heat/freezing, Animal Diagnostic Specimen)

Packing and Shipping

- Fill out USDA BSE Surveillance Submission Form (attaching bar code label) and USDA BSE Surveillance Data Collection Form (attaching bar code label) (see Appendix G for guidance on completing forms).
- Place labeled sample tubes into plastic bag with absorbent.
- Place plastic bag into clear bio-hazard bag (STP-741) and seal.
- Place this bag into white bio-hazard bag (STP-740) and seal.
- Place the white bag into your shipping box.
- Place frozen ice packs on top of the bag.
- Place completed USDA BSE Surveillance Submission Form and USDA BSE Surveillance Data Collection Form on top of inner Styrofoam lid.
- Seal box
- Place address shipping label on the box (supplied by local Federal veterinarian); addressed to appropriate laboratory conducting BSE testing for your state.
- Place the other required shipping labels on the box.
- Ship by overnight delivery with the Federal contract service.
- If shipping on a Friday, be sure to mark/label box for Saturday delivery.

Place sample tube on cold packs as soon as possible.

Do NOT freeze!

Sample packed in sample tube.

NVSL supplies a certified shipping box and all supplies need for shipping as BSE kits. To request additional BSE kits, fax a request to number noted below.

Samples contained in formaldehyde are exempt from requirements for diagnostic specimens. Formalin fixed samples should be sent only to NVSL and returned in the box with the absorbent material provided by NVSL.

If you need further assistance with shipping, you may contact the shipping department at:

National Veterinary Services Laboratories
1800 Dayton Avenue
Ames, IA 50010
Ph: (515) 663-7530
Fax: (515) 663-7378

Designated Laboratories for BSE Sample Submission

NOTE: All highly suspicious cases as defined in VS Memo 580.16 must be sent to NVSL.

State where sample collected	Designated laboratory
All states submitting formalin fixed tissues for IHC testing. This includes all submissions from Puerto Rico.	USDA, APHIS, VS, NVSL 1800 Dayton Ave. Ames, IA 50010 Steve Growen 888-273-6875 bsemailcases@aphis.usda.gov
Texas, Arkansas, Louisiana, New Mexico	Texas A&M University TVMDL Pathology Department 1 Sippel Road College Station, TX 77843 Dr. Levle Gayle 979-845-3414 gfearneyhouge@tvmdl.tamu.edu l-gayle@tvmdl.tamu.edu
Washington, Oregon, Idaho, Montana, Hawaii, Alaska,	Washington State University WADDL Animal Disease Diagnostic Laboratory Bustad Hall Room 155-N Pullman WA 99164-7034 Tim Bazzler 509-335-9696 baszlert@vetmed.wsu.edu
Georgia, Mississippi, Alabama, Tennessee, Virginia, North Carolina, South Carolina, Oklahoma, Florida	Athens Diagnostic Laboratory College of Veterinary Medicine University of Georgia Athens, GA 30602 Doris Miller 706-542-5568 BSE@vet.uga.edu
California, Arizona, Nevada	CAHFS-Thurman Bldg West Health Science Drive UC Davis Davis, CA 95616 Jackie Parker Tel 530-753-6352 Fax 530-754-5665

Colorado, Utah, Wyoming, Nebraska, South Dakota, North Dakota, Kansas, Missouri, Iowa, Illinois, Indiana, Kentucky, Ohio, West Virginia, Minnesota, Wisconsin (FSIS only)	Colorado State University Veterinary Diagnostic Laboratory 300 West Drake, Rm. E-100 Ft. Collins, CO 80526 Dr. Barbara Powers Tel 970-297-1281 barb.powers@colostate.edu
New York, Maine, Maryland, Massachusetts, New Hampshire, New Jersey, Vermont, Rhode Island, Delaware, Connecticut, Michigan, Pennsylvania	Cornell University Animal Health Diagnostic Laboratory College of Veterinary Medicine at Cornell Upper Tower Road Ithaca, NY 14853 Edward J. Dubovi 607-253-3900 tselab@cornell.edu
Wisconsin	WVDL – TSE Laboratory 6101 Mineral Point Rd Madison, WI 53705 Dr. Phil Bochsler 608-262-5432 tselab@wvdl.wisc.edu

The following states will be sending samples to one of five additional laboratories when they are on-line. Notification will be sent out and this table will be changed as these labs come on-line.

- Florida
- Kansas
- Kentucky
- Minnesota
- Missouri
- Ohio
- Pennsylvania (central and eastern)
- West Virginia

Proper Communication for Submitting Samples

It is essential to have secure and reliable communication among the individuals responsible for sample collection at collection locations, establishments' management, and NVSL or designated laboratories. Sample submitter – designated laboratory communication guidelines are as follows:

- The sample submitter will notify the appropriate laboratory (see pages 20-21) of incoming samples via facsimile, telephone, e-mail, or any other approved electronic method. This includes when electronic submission of the BSE Surveillance Submission Form is used. The information to be communicated will include the overnight contract delivery service tracking number, the collection site name and address, the unique Referral Number of the submission, and the number of samples. There is currently a dedicated e-mail box for notifying NVSL of incoming samples (bsemailcases@aphis.usda.gov). Sample submitters must accurately record all relevant information on the USDA BSE Surveillance Submission Form, USDA BSE Surveillance Submission Continuation Form if used, and on each of the USDA BSE Surveillance Data Collection Forms. Enter this information on the electronic version of these forms – either on a tablet PC or via the web-based forms – unless such electronic entry is impossible. Print a copy of the completed BSE Surveillance Submission Form, BSE Surveillance Submission Continuation Form if used, and on each BSE Surveillance Data Collection Form to accompany the samples shipped to the designated laboratory. Prepare four (4) copies of these completed forms for further distribution and filing (one for the submitter, one for the collection site, one for the VS Area Office, and one to be maintained with the identification devices). See Appendix G for instructions on completing the BSE Surveillance Submission Form and BSE Surveillance Data Collection Form. See Appendix H for instructions on using barcodes in shipping the sample.

- The paper version of the BSE Surveillance Submission Form has space to indicate the identification number for 20 animals. If additional animals are sampled, the sample submitter should submit a BSE Surveillance Submission Continuation Form listing the unique identification numbers for each additional animal.

- The sample submitter should verify, via the overnight contract delivery service tracking system, that the submission has been delivered to the designated laboratory. If the sample does not arrive as expected, the sample submitter should work with the delivery service to determine the location and delivery status of the sample.

Disposal

1. Disposal of carcasses and offal from sampled cattle – Dispose of carcasses and offal in compliance with Federal, State, and local laws. Acceptable options include the following, among others:
    - Refrigerate or freeze pending test results, then render or otherwise process after negative test results obtained (could include boning out carcass and holding the meat product for use in pet food or rendering materials and holding finished product).
    - Render at dedicated facilities, if available – render for non-animal feed use, such as biofuel or cement.
    - Bury in a landfill or on-the-farm.
    - Use alkaline digestion.
    - Incinerate.

2. Hides – Hides need not be disposed or held pending test results.

3. Sample disposal – Laboratories will dispose of samples using standard operating procedures.